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The 4′,4′-difluoro analog of 5′-noraristeromycin: A new structural prototype for possible antiviral drug development toward orthopoxvirus and cytomegalovirus

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Abstract—As a surrogate for 4′-hydroxy-5′-noraristeromycin and related carbocyclic nucleosides, an efficient, enantiodivergent synthetic route to both enantiomers of 5-(6-amino-9*H*-purin-9-yl)-3,3-difluorocyclopentane-1,2-diol (**6** and *ent*-**6**) has been developed from a common starting material ((+)-(1*R*,4*S*)-4-hydroxy-2-cyclopenten-1-yl acetate, **10**). Both compounds were assayed versus a series of viruses. The only response found was for compound **6** toward vaccinia and cowpox (EC_{50} of 143 and 94 μM, respectively) and human cytomegalovirus (EC_{50} of 6.2 μM). Both compounds were non-cytotoxic. While not as active as cidofovir toward the orthopox viruses and ganciclovir toward cytomegalovirus, compound **6** offers a new structural prototype upon which to build for uncovering new agents effective against these viral types.

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1. Introduction

In our laboratory, we have found that 5′-noraristeromycin (**1**) (Fig. 1) displays significant biological activity due to its apparent inhibition of *S*-adenosyl-L-homocysteine (AdoHcy) hydrolase.[1] The 4′-hydroxy epimer of **1** (i.e., **2**) has an antiviral profile similar to **1**.[2] Blending of these structural components into a derivative possessing both hydroxyls (see **3**) is not reasonable because of the instability expected for what is actually the hydrated form of ketone **4**, which, in turn, is one member of a keto/enolic tautomeric mixture containing other cyclopentyl keto centers (via, e.g., **5**).[3]

However, there is considerable precedence that replacement of a hydroxyl substituent with a fluorine atom renders the new compound with biological properties similar, or superior, to the parent compound.[4] It was with this in mind that compound **6** became a target substitute compound for **3** in our search for antiviral agents based on the 5′-noraristeromycin prototype structure. Compound **6** was also sought as a surrogate for **7**, an isomer of β-D-erythrofuranosyladenine,[5] which is not likely to exist as shown as a consequence of it being a cyclic hemiacetal (from **8**). The structural analogy between compounds **6** and **7** can be found in the extensive medicinal and biochemical (including structural) literature whereby the difluoromethylene (CF_2) unit has served as an isosteric and isoelectronic (isopolar) replacement for an ethereal oxygen.[6]

Our interest in compound **6** (as represented by **7**) was also based on its resemblance to (i) the isonucleosides of Nair and co-workers[7] and (ii) the ribofuranose-derived antiviral nucleosides wherein the furanose oxygen and ring carbons are interchanged.[8] Finally, the potential of compound **6** was correlated to biologically active nucleosides possessing a *gem*-difluoro site in their ribofuranose substitutent (as in, e.g., gemcitabine **9**).[9]

The results of this effort are described.

1.1. Chemistry

A retrosynthetic pathway to compound **6** and its enantiomer (*ent*-**6**) suggested that (+)-(1*R*,4*S*)-4-hydroxy-2-cyclopenten-1-yl acetate (**10**)[10] could serve as a common

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Keywords: Enantiodivergent; Adenine; Carbocyclic nucleosides; Antiviral.

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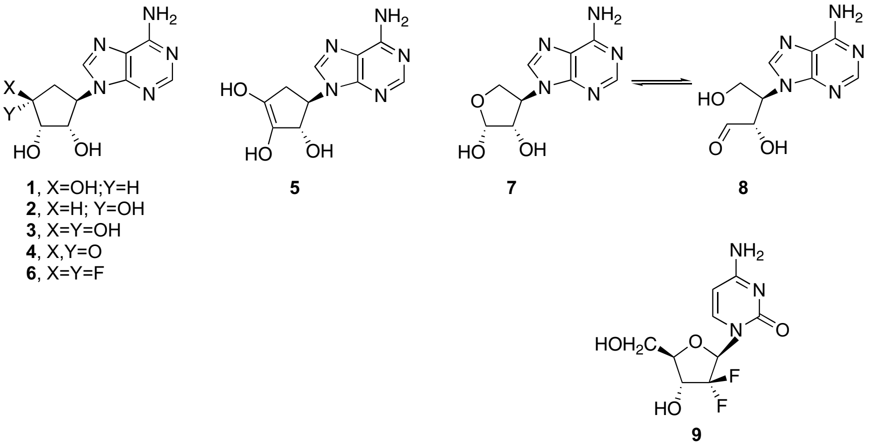


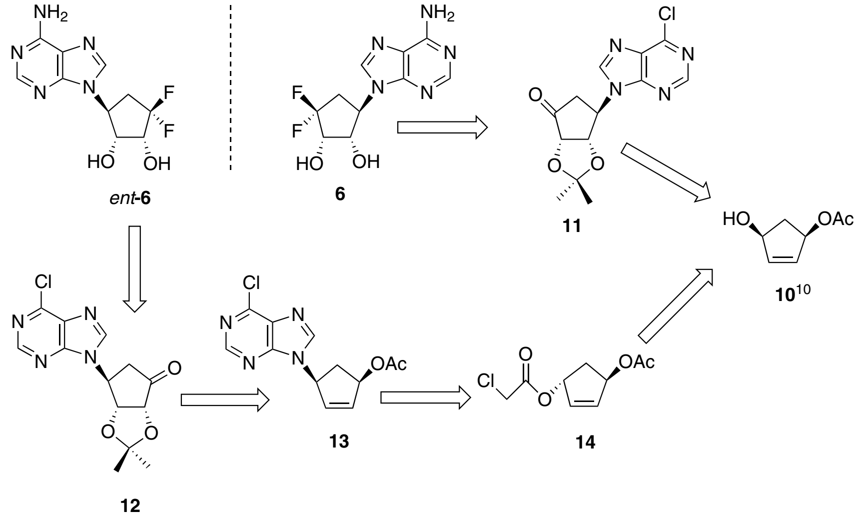
Figure 1.

precursor (Scheme 1). With that established, the synthesis of compound **6** (Scheme 2) began with the convenient conversion of **10** into **15**.[11] Oxidation of **15** with pyridinium chlorochromate resulted in the formation of the key intermediate ketone **11** without accompanying elimination of the base, which we had encountered previously when seeking **11** via other means (e.g., when the base was adenine or an N-6 protected adenine). Incorporation of the requisite geminal difluoro moiety was accomplished by the reaction of ketone **11** with diethylaminosulfur trifluoride (DAST) to afford **16** (Scheme 2). The ^{19}F NMR spectrum of **16** was consistent with the presence of two diastereotopic fluorine atoms that showed two signals at δ −95.46 (dddd) and −106.84 (dt) ppm with a characteristic geminal coupling constant of $J_{F,F} = 263.75$ Hz. The quaternary carbon bearing the two fluorine atoms appeared as a doublet of doublets in the ^{13}C NMR spectrum at δ 127.47 ppm with $^1J_{C,F(1)} = 251.12$ and $^1J_{C,F(2)} = 265.37$ Hz, which is characteristic of the CF_2 group. Ammonolysis of **16** (to **17**) followed by deprotection with Dowex H^+ furnished the target compound **6**.

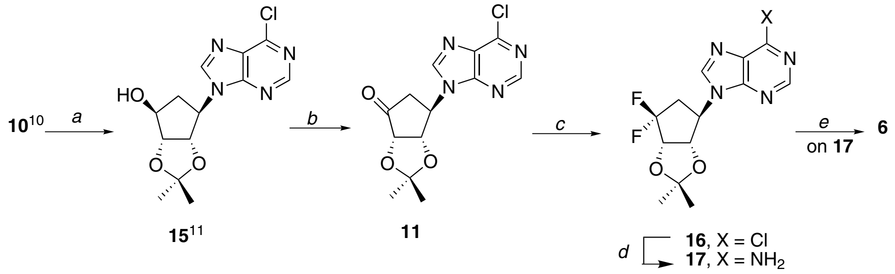
To achieve *ent*-**6** via a similar scheme required the synthesis of **12** (the enantiomer of **11**). This began with treatment of **10** with chloroacetic acid under Mitsunobu conditions[12] at room temperature to afford chloroacetate ester **14** (Scheme 3). Selective cleavage[13] of the chloroacetate ester moiety in the presence of the acetate ester group of **14** using thiourea yielded the inverted hydroxyester **18**.[14] When **18** was subjected to a second Mitsunobu reaction with 6-chloropurine, compound **13** resulted. Dihydroxylation of **13** to **19** was followed by isopropylidenation to **20**. Deacetylation of **20** produced **21**, which, on oxidation with pyridinium chlorochromate, furnished **12**. *Ent*-**6** was then obtained (Scheme 4) from **12** through the same sequence of reactions as depicted for the preparation of **6** in Scheme 2.

1.2. Antiviral results

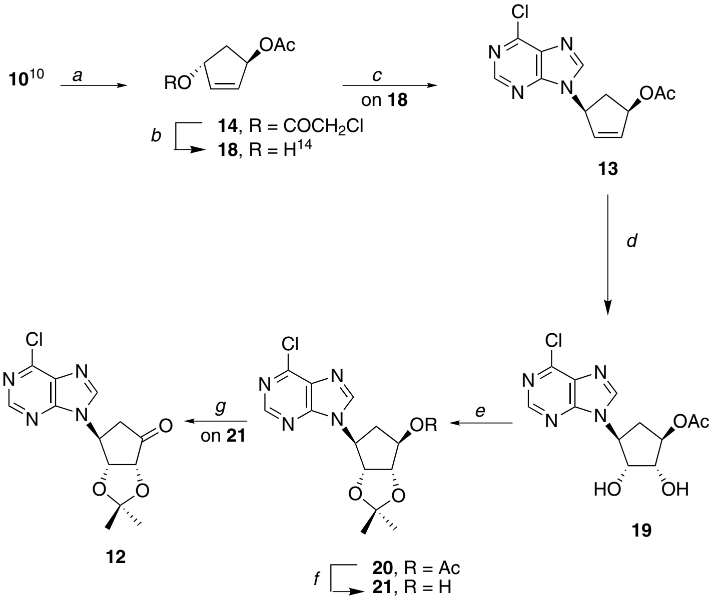
Compounds **6** and *ent*-**6** were subjected to broad antiviral analysis.[15] Only compound **6** displayed any activity and even that was marginal toward vaccinia virus,



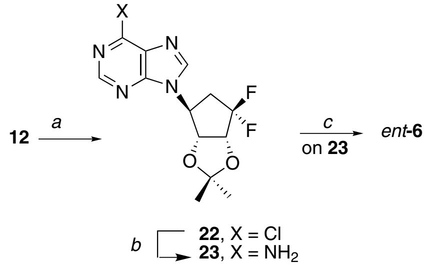
Scheme 1.



Scheme 2. Reagents and conditions: (a) Ref. 11; (b) PCC, CH_2Cl_2 (71%); (c) DAST, CH_2Cl_2 (85%); (d) NH_3, MeOH, 120 °C (86%); (e) Dowex H^+ (87%).



Scheme 3. Reagents and conditions: (a) PPh_3, DIAD, THF, $ClCH_2CO_2H$ (90%); (b) thiourea, $NaHCO_3$, EtOH (71%); (c) PPh_3, DIAD, THF, 6-chloropurine (76%); (d) OsO_4, NMO, THF/H_2O/acetone (8:1:1) (85%); (e) 2,2-dimethoxypropane, acetone, *p*-TsOH (87%); (f) KCN, MeOH/H_2O (87%); (g) PCC, CH_2Cl_2 (71%).



Scheme 4. Reagents and conditions: (a) DAST, CH_2Cl_2 (84%); (b) NH_3, MeOH, 120 °C (86%); (c) Dowex H^+ (87%).

cowpox, and human cytomegalovirus (HCMV) when compared to positive controls. In this regard, the EC_{50} values of 143 and 94 μM for compound **6** against vaccinia and cowpox, respectively, were 5-fold (30 μM) and 3-fold (36 μM) higher than cidofovir (CDV) for the same viruses. The selectivity index (SI) for compound **6** in these assays (determined by dividing the CC_{50} by the EC_{50}) was >2.5 for vaccinia and >3.9 for cowpox compared to >10.6 and >8.8 for CDV.

Slightly more promising antiviral potential was observed for compound **6** against HCMV (EC_{50} of 6.2 μM; SI of >58.5), although again, in this instance, the positive control ganciclovir (GCV) (EC_{50} of 2.6 μM; SI of >151) was more active.

There was little evidence of cellular cytotoxicity for either compound, but compound **6** exhibited some cell proliferation (IC_{50} of 84 μM, HFF cells).

2. Experimental

2.1. General methods

Melting points were recorded on a Meltemp II melting point apparatus and are uncorrected. Atlantic Microlab, Inc., Norcross, GA performed combustion analyses. The NMR spectra were recorded on a Bruker AC 250 spectrometer operated at 250 MHz for ^{1}H and ^{19}F and 62.5 MHz for ^{13}C and are referenced to internal tetramethylsilane (TMS) at 0.0 ppm. The spin multiplicities are indicated by the symbols s (singlet), d (doublet), t (triplet), m (multiplet), and br (broad). Optical rotations were measured on a Jasco P-1010 polarimeter. Reactions were monitored by thin-layer chromatography (TLC) using 0.5-mm Whatman Diamond silica gel 60-F_{254} precoated plates with visualization by irradiation with a Mineralight UVGL-25 lamp. Yields refer to chromatographically and spectroscopically (^{1}H and ^{13}C NMR) homogeneous materials.

2.1.1. (2*S*,3*S*,4*R*)-4-(6-Chloro-9*H*-purin-9-yl)-2,3-(isopropylidenedioxy)cyclopent-1-one (11). A solution of **15**[11] (910 mg, 2.93 mmol) in dry CH_2Cl_2 (25 mL) was added slowly to a stirred suspension of pyridinium chlorochromate (1.26 g, 5.86 mmol) in dry CH_2Cl_2 (50 mL) at room temperature. After 24 h, Et_2O (150 mL) was added and the reaction mixture was filtered through silica gel. The filtrate was concentrated in vacuo. The residue was purified via column chromatography eluting with hexanes/EtOAc (1:3) to afford **11** (643 mg, 71%) as a solid: mp 112–113.2 °C. ^{1}H NMR ($CDCl_3$) δ 1.30 (s, 3H), 1.60 (s, 3H), 2.84 (d, 1H, $J = 19.37$ Hz), 3.34 (dd, 1H, $J = 9.64, 19.37$ Hz), 4.91 (d, 1H, $J = 5.63$ Hz), 5.04 (d, 1H, $J = 5.76$ Hz), 5.18, (dd, 1H, $J = 2.81, 9.62$ Hz), 8.21 (s, 1H), 8.74 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 24.89, 26.89, 40.63, 55.70, 79.63, 82.39, 113.38, 131.74, 144.61, 147.25, 151.71, 152.13, 200.48. Anal. Calcd for $C_{13}H_{13}ClN_4O_3$: C, 50.58; H, 4.24; N, 18.15. Found: C, 50.47; H, 4.14; N, 18.21.

2.1.2. (1*S*,2*S*,5*R*)-5-(6-Chloro-9*H*-purin-9-yl)-3,3-difluoro-1,2-(isopropylidenedioxy)cyclopentane (16). To the solution of ketone **11** (500 mg, 1.62 mmol) in CH_2Cl_2 (50 mL), DAST (0.54 mL, 4.05 mmol) was added at room temperature and the mixture was refluxed for 16 h. The reaction mixture was cooled to room temperature and quenched with a saturated solution of $NaHCO_3$. The organic layer was separated and the aqueous layer extracted with CH_2Cl_2 (3 × 60 mL). The combined organic layers were washed with H_2O (75 mL), dried (anhydrous Na_2SO_4), and evaporated under reduced pressure. The residue was chromatographed (hexanes/EtOAc, 1:1) to produce **16** (459 mg, 85%) as a foam. ^{1}H NMR ($CDCl_3$) δ 1.40 (s, 3H), 1.63 (s, 3H), 2.86–3.16 (m, 2H), 4.85 (t, 1H, $J = 6.15$ Hz), 5.15 (d, 2H, $J = 6.57$ Hz), 8.17 (s, 1H), 8.78 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 24.68, 26.35, 36.98 (t, $^2J_{C,F} = 24.68$ Hz), 57.17 (d, $^3J_{C,F} = 7.38$ Hz), 80.23 (dd, $^3J_{C,F} = 18.93$, 34.50 Hz), 83.08 (d, $^2J_{C,F} = 38.45$ Hz), 114.62, 127.47 (dd, $^1J_{C,F} = 251.12$, 265.37 Hz), 131.88, 143.64, 151.46, 151.65, 152.25. ^{19}F NMR ($CDCl_3$) δ −106.84 (dt, $J = 256.50$, 6.75 Hz), −95.46 (dddd, $J = 256.50$, 36.50, 24.25, 11.75 Hz). Anal. Calcd for $C_{13}H_{13}ClF_2N_4O_2$: C, 47.21; H, 3.96; N, 16.94. Found: C, 47.19; H, 4.13; N, 16.74

2.1.3. (1*S*,2*S*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,3-difluoro-1,2-(isopropylidenedioxy)cyclopentane (17). A solution of **16** (400 mg, 1.29 mmol) in saturated methanolic ammonia (75 mL) was sealed in a Parr stainless steel vessel and heated at 120 °C for 24 h. After cooling to room temperature, the solvent was evaporated and the residue purified via flash chromatography (EtOAc/MeOH, 49:1) to give **17** (325 mg, 86%) as a white solid: mp 163.5–164.1 °C. ^{1}H NMR (DMSO-d_6) δ 1.24 (s, 3H), 1.52 (s, 3H), 2.85–3.01 (m, 2H), 4.87–4.98 (m, 2H), 5.18 (d, 1H, $J = 6.48$ Hz), 7.32 (br s, 2H), 8.18 (s, 1H), 8.23 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 24.74, 26.01, 37.30 (t, $^2J_{C,F} = 20.13$ Hz), 59.73, 79.68 (t, $^3J_{C,F} = 8.41$ Hz), 82.38 (d, $^2J_{C,F} = 19.12$ Hz), 113.58, 118.90, 126.31 (dd, $^1J_{C,F} = 247.82$, 261.21 Hz), 139.20, 149.31, 152.56, 156.08. ^{19}F NMR (DMSO-d_6) δ −102.87 (dt, $J =$ 255.50, 13.65 Hz), −94.51 (dddd, $J = 255.50$, 27.50, 14.75, 6.75 Hz). Anal. Calcd for $C_{13}H_{15}F_2N_5O_2$: C, 50.16; H, 4.86; N, 22.50. Found: C, 50.02; H, 4.96; N, 22.74.

2.1.4. (1*S*,2*S*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,3-difluorocyclopentane-1,2-diol (6). To the solution of **17** (250 mg, 0.92 mmol) in MeOH (60 mL), Dowex 50 × 8 resin (8.5 g) was added and the mixture refluxed for 10 h. After removing the solvent, the residue was loaded onto a Dowex resin column and the product eluted with 14% NH_4OH. The fractions containing product were combined and evaporated under reduced pressure. The resultant residue was purified via flash chromatography (EtOAc/MeOH, 17:3) to afford 190 mg (87%) of **6** as a white crystalline solid: mp 206.5–206.7 °C; $[\alpha]_D^{22}$ −74.2 (*c* 0.12, MeOH). ^{1}H NMR (DMSO-d_6) δ 2.66–2.82 (m, 2H), 4.02 (br s, 1H), 4.61 (br s, 1H), 4.90 (q, 1H, $J = 9.27$ Hz), 5.47 (d, 1H, $J = 6.75$ Hz), 5.94 (d, 1H, $J = 5.01$ Hz), 7.23 (br s, 2H), 8.12 (s, 1H), 8.16 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 36.44 (t, $^2J_{C,F} = 24.81$ Hz), 57.17 (t, $^3J_{C,F} = 4.13$ Hz), 72.54 (t, $^3J_{C,F} = 9.34$ Hz), 72.98 (d, $^2J_{C,F} = 18.81$ Hz), 119.42, 125.91 (dd, $^1J_{C,F} =$ 246.87, 258.13 Hz), 140.56, 149.47, 152.26, 156.05. ^{19}F NMR (DMSO-d_6) δ −107.34 (dt, $J = 258.12$, 10.40 Hz), −96.00 (dddd, $J = 258.12$, 26.21, 13.14, 7.10 Hz). Anal. Calcd for $C_{10}H_{11}F_2N_5O_2 \cdot 0.25H_2O$: C, 43.56; H, 4.20; N, 25.40. Found: C, 43.34; H, 4.28; N, 25.57.

2.1.5. (1*R*,4*R*)-4-Acetoxycyclopent-2-ene-1-yl chloroacetate (14). A solution of triphenylphosphine (6.00 g, 22.9 mmol) in dry THF (70 mL) was cooled to −20 °C and diisopropyl azodicarboxylate (4.5 mL, 22.9 mmol) added over a period of 10 min. This mixture was stirred at −20 °C for 20 min to yield a white precipitate of the triphenylphosphine–diisopropyl azodicarboxylate complex. To this latter complex as a suspension were added a solution of **10**[10] (2.5 g, 17.61 mmol) in dry THF (40 mL) and chloroacetic acid (2.16 g, 22.9 mmol). The cooling bath was removed, and the reaction mixture was stirred at room temperature for 2 h. After evaporation of the reaction mixture to dryness, the residue was purified via flash chromatography (hexanes/EtOAc, 9:1) to afford **14** (3.45 g, 90%) as a colorless liquid. ^{1}H NMR ($CDCl_3$) δ 1.25–1.34 (m, 1H), 2.06 (s, 3H), 2.25–2.31 (m, 1H), 4.08 (s, 2H), 5.78–5.81 (m, 1H), 5.82–5.90 (m, 1H), 6.12–6.20 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 20.90, 37.02, 40.81, 77.96, 80.14, 134.30, 136.36, 167.02, 170.61. Anal. Calcd for $C_9H_{11}ClO_4$: C, 49.44; H, 5.07. Found: C, 49.12; H, 5.33.

2.1.6. (1*R*,4*R*)-4-Hydroxycyclopent-2-ene-1-yl acetate (18). To a solution of **14** (2.28 g, 10.43 mmol) in absolute EtOH (60 mL) were added thiourea (1.03 g, 13.56 mmol) and $NaHCO_3$ (1.14 g, 13.56 mmol) and this reaction mixture was heated at 70–75 °C for 4 h. After cooling to room temperature, it was filtered over a Celite pad; the pad was washed with EtOAc and the filtrate evaporated under reduced pressure. The residue was dissolved in EtOAc. This solution was washed with distilled H_2O (2×75 mL) and evaporated. The resultant residue was purified by flash chromatography (EtOAc/hexanes, 2:3) to give **18** (1.06 g, 71%) as a colorless oil, which had spectral data identical to that reported in the literature.[14]

2.1.7. (1*R*,4*S*)-4-(6-Chloro-9*H*-purin-9-yl)cyclopent-2-ene-1-yl acetate (13). To a cooled (−20 °C) solution of triphenylphosphine (2.35 g, 8.96 mmol) in dry THF (50 mL), diisopropyl azodicarboxylate (1.76 mL, 8.96 mmol) was added over a period of 10 min. This mixture was stirred at −20 °C for 20 min to yield a white precipitate of triphenylphosphine–diisopropyl azodicarboxylate complex. To this latter complex as a suspension were added a solution of **18** (910 mg, 6.4 mmol) in dry THF (20 mL) and 6-chloropurine (1.38 g, 8.96 mmol). The cooling bath was removed, and the reaction mixture was stirred at room temperature for 12 h. After evaporation of the reaction mixture to dryness, the residue was purified via flash chromatography (hexanes/EtOAc, 3:2) to afford **13** (1.36 g, 76%) as a white solid: mp 129.5–130.8 °C. ^1H NMR ($CDCl_3$) δ 2.01 (dt, 1H, $J = 15.11, 5.6$ Hz), 2.08 (s, 3H), 3.13–3.26 (m, 1H), 5.83 (t, 2H, $J = 7.20$ Hz), 6.32 (d, 1H, $J = 5.52$ Hz), 6.45 (d, 1H, $J = 5.54$ Hz), 8.27 (s, 1H), 8.76 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 20.83, 38.35, 57.34, 76.65, 131.42, 133.30, 136.18, 143.44, 150.56, 151.13, 151.65, 170.02. Anal. Calcd for $C_{12}H_{11}ClN_4O_2$: C, 51.72; H, 3.98; N, 20.10. Found: C, 52.07; H, 4.17; N, 19.98.

2.1.8. (1*R*,2*R*,3*R*,4*S*)-4-(6-Chloro-9*H*-purin-9-yl)-2,3-dihydroxycyclopent-1-yl acetate (19). To a solution of **13** (2.2 g, 7.89 mmol) in THF/H_2O/acetone (8:1:1, 60 mL) were added 50% *N*-methylmorpholine *N*-oxide (3.7 mL, 15.78 mmol) and OsO_4 (50 mg) and the solution stirred at room temperature overnight. The solvents were removed under reduced pressure. The residue was co-evaporated with EtOH (3×75 mL), and the resultant material was purified by column chromatography (EtOAc/hexanes, 4:1) to afford 2.10 g (85%) of **19** as a white solid: mp 172.9–173.7 °C. ^1H NMR ($CDCl_3$) δ 1.23–1.32 (br s, 1H), 2.14 (s, 3H), 2.37 (m, 1H), 2.95–3.20 (m, 1H), 4.01–4.14 (m, 1H), 4.30 (br s, 1H), 4.84 (t, 1H, $J = 5.80$ Hz), 5.00–5.10 (m, 2H), 8.46 (s, 1H), 8.66 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 20.94, 32.45, 60.34, 74.23, 74.71, 76.22, 131.30, 145.68, 150.16, 151.42, 151.50, 170.60. Anal. Calcd for $C_{12}H_{13}ClN_4O_4$: C, 46.09; H, 4.19; N, 17.92. Found: C, 46.33; H, 4.17; N, 17.69.

2.1.9. (1*R*,2*R*,3*R*,4*S*)-4-(6-Chloro-9*H*-purin-9-yl)-2,3-(isopropylidenedioxy)-cyclopent-1-yl acetate (20). To a solution of **19** (1.64 g, 5.24 mmol) in dry acetone (30 mL) containing 2,2-dimethoxypropane (20 mL) was added *p*-toluenesulfonic acid (200 mg) and this reaction mixture was stirred at room temperature for 12 h. The mixture was then neutralized with NH_4OH and solvent removed under reduced pressure; the residue was co-evaporated with MeOH (3×60 mL). The resultant material was then purified by column chromatography (EtOAc/hexanes, 2:3) to give 1.61 g (87%) of **20** as a white solid: mp 150.6–151.4 °C. ^1H NMR ($CDCl_3$) δ 1.32 (s, 3H), 1.57 (s, 3H), 2.11 (s, 3H), 2.50 (dt, 1H, $J = 3.43, 15.20$ Hz), 2.87–2.99 (m, 1H), 4.83 (d, 1H, $J = 5.84$ Hz), 5.14 (t, 2H, $J = 11.25$ Hz), 5.31 (t, 1H, $J = 3.63$ Hz), 8.31 (s, 1H), 8.79 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 20.95, 24.22, 26.56, 34.56, 61.25, 76.72, 84.06, 84.21, 112.53, 132.71, 144.10, 151.07, 151.80, 151.95, 169.45. Anal. Calcd for $C_{15}H_{17}ClN_4O_4$: C, 51.07; H, 4.86; N, 15.88. Found: C, 51.02; H, 4.96; N, 15.99.

2.1.10. (1*S*,2*R*,3*S*,4*R*)-1-(6-Chloro-9*H*-purin-9-yl)-4-hydroxy-2,3-(isopropylidenedioxy)cyclopentane (21, *ent*-15). To a solution of **20** (1.5 g, 4.25 mmol) in MeOH (50 mL) was added four drops of H_2O and catalytic amount of KCN (50 mg). This mixture was stirred at room temperature for 2.5 h. After evaporation of the reaction mixture to dryness, the residue was purified via flash chromatography (hexanes/EtOAc, 1:1) to afford **21** (1.15 g, 87%) as a white solid: mp 162.3–163.1 °C. ^1H NMR ($CDCl_3$) δ 1.32 (s, 3H), 1.54 (s, 3H), 2.27 (d, 1H, $J = 15.48$ Hz), 2.88 (m, 1H), 4.40 (br s, 1H), 4.59 (d, 1H, $J = 4.57$ Hz), 4.74 (d, 1H, $J = 5.44$ Hz), 4.91 (d, 1H, $J = 5.47$ Hz), 5.12 (d, 1H, $J = 8.93$ Hz), 8.58 (s, 1H), 8.77 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 24.22, 26.71, 36.85, 62.40, 76.07, 86.27, 87.05, 111.78, 131.62, 145.82, 150.94, 151.50, 151.75. Anal. Calcd for $C_{13}H_{15}ClN_4O_3$: C, 50.25; H, 4.87; N, 18.03. Found: C, 50.06; H, 5.15; N, 18.21.

2.1.11. (2*R*,3*R*,4*S*)-4-(6-Chloro-9*H*-purin-9-yl)-2,3-(isopropylidenedioxy)cyclopent-1-one (12, *ent*-11). Following the same procedure used in the preparation of **11**, **21** led to **12** (71%) as a solid: mp 112.5–113.3 °C. The spectral data (^1H NMR and ^{13}C NMR) were identical with that of **11**. Anal. Calcd for $C_{13}H_{13}ClN_4O_3$: C, 50.58; H, 4.24; N, 18.15. Found: C, 50.51; H, 4.17; N, 18.18.

2.1.12. (1*R*,2*R*,5*S*)-5-(6-Chloro-9*H*-purin-9-yl)-3,3-difluoro-1,2-(isopropylidenedioxy)cyclopentane (22, *ent*-16). Following the same procedure used in the preparation of **16**, **12** led to **22** (84%) as a foam. The spectral data (^1H, ^{13}C, and ^{19}F NMR) was identical to **16**. Anal. Calcd for $C_{13}H_{13}ClF_2N_4O_2$: C, 47.21; H, 3.96; N, 16.94. Found: 47.02; H, 4.15; N, 16.81.

2.1.13. (1*R*,2*R*,5*S*)-5-(6-Amino-9*H*-purin-9-yl)-3,3-difluoro-1,2-(isopropylidenedioxy)cyclopentane (23, *ent*-17). As for the preparation of **17**, ammonolysis of **22** provided **23** in identical yield (86%) as a white solid: mp 164.5–164.9 °C. The spectral data (^1H, ^{13}C, and ^{19}F NMR) were identical with that of **17**. Anal. Calcd for $C_{13}H_{15}F_2N_5O_2$: C, 50.16; H, 4.86; N, 22.50. Found: C, 50.07; H, 4.98; N, 22.68.

2.1.14. (1*R*,2*R*,5*S*)-5-(6-Amino-9*H*-purin-9-yl)-3,3-difluorocyclopentane-1,2-diol (*ent*-6). Employing the same procedure used to produce **6**, compound ent-**6** was obtained in identical yield (87%) as a crystalline solid from **23**: mp 206.5–206.8 °C; $[\alpha]_D^{22.0}$ +74.8 (*c* 0.13, MeOH). The ^1H, ^{13}C, and ^{19}F NMR spectra were identical with that of **6**. Anal. Calcd for $C_{10}H_{11}F_2N_5O_2$: C, 44.30; H, 4.10; N, 25.83. Found: C, 44.11; H, 4.13; N, 25.74.

2.1.15. Virus pools, media, and cells. Vaccinia virus (VV) strain Copenhagen and cowpox virus (CV) strain Brighton stock pools prepared in Vero cells were obtained from Dr. John Huggins of USAMRIID, Frederick, MD, and were diluted to prepare the working stocks. Human cytomegalovirus (HCMV) strain AD169 was received from American Type Culture Collection, Manassas, VA, and were propagated in human foreskin fibroblast (HFF) cells. These cells were prepared as primary cultures from freshly obtained newborn human foreskins (University of Alabama at Birmingham or Brookwood Hospital, Birmingham, AL) as soon as possible after circumcision. Culture medium was Eagle's minimal essential medium (MEM) containing 10% fetal bovine serum (FBS) and standard concentrations of L-glutamine, penicillin, and gentamic.

2.2. Antiviral assays

2.2.1. Plaque reduction assay for vaccina virus and cowpox virus using a semi-solid overlay. Two days prior to use, HFF cells were plated onto six-well plates and incubated at 37 °C with 5% CO_2 and 90% humidity. On the day of assay, the drug was made up of twice the desired concentration in 2× MEM with 10% FBS and diluted serially 1:5 in 2× MEM to provide final concentrations of drug ranging from 100 to 0.032 μg/mL. The virus to be used was diluted in MEM containing 10% FBS to a desired concentration, which would give 20–30 plaques per well. The media was then aspirated from the wells and 0.2 mL of virus added to each well in triplicate with 0.2 mL of media being added to drug and cell control wells. The plates were incubated for 1 h with shaking every 15 min. After the incubation period, an equal amount of 1% agarose was added to an equal volume of each drug dilution. The drug/agarose mixture was added to each well in 2 mL volumes and the plates incubated for 3 days, after which the cells were stained with 0.02% solution of neutral red in PBS. After a 5–6 h incubation period, the stain was aspirated, and plaques counted using a stereomicroscope at 10× magnification. The MacSynergy II, version 1 computer program was used to calculate the 50% effective concentration (EC_{50}) values.

2.2.2. Plaque reduction assay for HCMV using liquid medium overlay. The procedure for the liquid overlay plaque assay was similar to that of the agarose overlay in that the procedure for adding the virus was the same. The drugs were made up of the desired concentrations in MEM with 2% FBS and added as 2 mL/well. Additional medium without new drug was added on day five and the plates were allowed to incubate for a total of 8 days. At the end of the incubation period, 2 mL of a 0.02% neutral red in PBS was added to each well and incubated for 6 h. The liquid was then aspirated off and plaques counted using a stereomicroscope at 10× magnification. The 50% effective concentration (EC_{50}) was calculated using the software indicated previously.

2.3. Cytotoxicity assays

2.3.1. Neutral red uptake assay. Twenty-four hours prior to assay, HFF cells were plated onto 96-well plates at a concentration of 2.5×10^4 cells per well. After 24 h, the media was aspirated and 125 μL of each drug concentration in MEM with 2% FBS was added to the first row of wells and then diluted serially 1:5 using the Beckman BioMek Liquid Handling System. Final drug concentrations ranged from 100 to 0.032 μg/mL. The plates were incubated for 7 days in a CO_2 incubator at 37 °C. After incubation, the media/drug was aspirated and 200 μL/well of 0.01% neutral red in PBS was added and incubated for 1 h. The dye was aspirated and the cells were washed with PBS using a Nunc Plate Washer. After removing the PBS, 200 μL/well of 50% EtOH/1% glacial AcOH (in H_2O) was added. The plates were placed on a rotary shaker for 15 min and the optical densities read at 540 nm on a Bio-tek plate reader. The 50% cytotoxic concentration of drug (CC_{50}) was calculated using the software indicated previously.

2.3.2. Cell proliferation assay. Twenty-four hours prior to assay, HFF cells were seeded in six-well plates at a concentration of 2.5×10^4 cells per well in MEM containing 10% FBS. On the day of the assay, drugs were diluted serially in MEM containing 10% FBS at increments of 1:5 covering a range from 100 to 0.03 μg/mL. For drugs, which were solubilized in DMSO, control wells received MEM containing 1% DMSO. The medium from the wells was aspirated and 2 mL of each drug concentration was then added to each well. The plates were incubated in a CO_2 incubator at 37 °C for 3 days. At the end of this time, the media/drug solutions were removed and the cells washed. One milliliter of 0.25% trypsin was added to each well and incubated until the cells started to detach from the plate. Vigorous up and down pipetting was used to break up the cell suspension and 0.2 mL of the mixture was added to 9.8 mL of Isoton III and counted using a Coulter Counter. Each sample was counted three times with two replicate wells per sample. The 50% inhibitory concentration (IC_{50}) was calculated using the software indicated previously.

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References and notes

1. Siddiqi, S. M.; Chen, X.; Schneller, S. W.; Ikeda, S.; Snoeck, R.; Andrei, G.; Balzarini, J.; De Clercq, E. *J. Med. Chem.* **1994**, *37*, 551–554.
2. Siddiqi, S. M.; Chen, X.; Schneller, S. W.; Ikeda, S.; Snoeck, R.; Andrei, G.; Balzarini, J.; De Clercq, E. *J. Med. Chem.* **1994**, *37*, 1382–1384.
3. Yin, X.-q.; Schneller, S. W. *Tetrahedron* **2004**, *60*, 3451–3455.
4. For two recent references see: (a) Chong, Y.; Gumina, G.; Mathew, J. S.; Schinazi, R. F.; Chu, C. K. *J. Med. Chem.* **2003**, *46*, 3245–3256; (b) He, J.; Mikhailopulo, I.; Seela, F. *J. Org. Chem.* **2003**, *68*, 5519–5524.
5. (a) Wu, X.; Guntha, S.; Ferencic, M.; Krishnamurthy, R.; Eschenmoser, E. *Org. Lett.* **2002**, *4*, 1279–1282; (b) Herdewijn, P. *Angew. Chem. Int. Ed.* **2001**, *40*, 2249–2251.
6. (a) Blackburn, G. M.; Eckstein, F.; Kent, D. E.; Perrée, T. D. *Nucleosides Nucleotides* **1985**, *4*, 165–167; (b) Halazy, S.; Ehrhard, A.; Danzin, C. *J. Am. Chem. Soc.* **1991**, *113*, 315–317; (c) Berkowitz, D. B.; Eggen, M.; Shen, Q.; Sloss, D. G. *J. Org. Chem.* **1993**, *58*, 6174–6176; (d) Kim, C. U.; Luh, B. Y.; Misco, P. F.; Bronson, J. J.; Hitchcock, M. J. M.; Ghazzouli, I.; Martin, J. C. *J. Med. Chem.* **1990**, *33*, 1207–1213; (e) Yang, Y.-Y.; Meng, W.-D.; Qing, F.-L. *Org. Lett.* **2004**, *6*, 4257–4259.
7. (a) Nair, V. In *Recent Advances in Nucleosides: Chemistry and Chemotherapy*; Chu, C. K., Ed.; Elsevier: Amsterdam, 2001; pp 149–166; (b) Nair, V.; Sosnouski, D. S.; Zhu, Q. M. *Nucleosides Nucleotides Nucleic Acids* **2001**, *20*, 735–738.
8. (a) Tam, S.; Holman, M.; Huryn, D.; Cislo, A. *Nucleosides Nucleotides* **1991**, *10*, 245–248; (b) Bednarski, K.; Dixit, D. M.; Wang, W.; Evans, C. A.; Jin, H.; Yuen, L.; Manour, T. S. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2667–2672.
9. (a) Bunn, P. A.; Kelly, K. *Clin. Cancer Res.* **1998**, *4*, 1087–1100; (b) Gmeiner, W. H. *Biopolymers* **2002**, *65*, 180–189.
10. Siddiqi, S. M.; Chen, X.; Schneller, S. W. *Nucleosides Nucleotides* **1993**, *12*, 267–278.
11. Hillman, J. M. L.; Roberts, S. M. *J. Chem. Soc., Perkin Trans. 1* **1997**, 3601–3608.
12. Mitsunobu, O. *Synthesis* **1981**, 1–28.
13. Naruto, M.; Ohno, K.; Naruse, N.; Takeuchi, H. *Tetrahedron Lett.* **1979**, *20*, 251–254.
14. Sugai, T.; Mori, K. *Synthesis* **1988**, *1*, 19–22.
15. Viruses subjected to **6** and *ent*-**6** were herpes simplex virus 1 and 2, human cytomegalovirus, varicella zoster virus, Epstein-Barr virus, vaccinia virus, cowpox virus, respiratory syncytial virus, Punta Toro virus, Venezuelan equine encephalitis, yellow fever, hepatitis B and C, coxsackie B4, vesicular stomatitis, parainfluenza-3, reovirus-1, and Sindbis.[1,16]
16. For leading references on the procedures used for the other viral assays see: (a) Rajappan, V. P.; Schneller, S. W.; Williams, S. L.; Kern, E. R. *Bioorg. Med. Chem.* **2002**, *10*, 883–886; (b) Seley, K. L.; Schneller, S. W.; Korba, B. *Nucleosides Nucleotides* **1997**, *16*, 2095–2099, and; (c) http://www.usu.edu/iar/Brochure/brochure.html (February 20, 2005).